Antral Follicle Counting in Predicting the Retrieved Oocyte Number After Ovarian Hyperstimulation

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Purpose: *To evaluate the antral follicle (AF) counting in predicting the outcome after controlled ovarian hyperstimulation (COH) and IVF-ET.*
Methods: *Infertile women who accepted the COH and IVF-ET were included prospectively. Day-3 AF number was determined by transvaginal sonography. They were divided into three groups: Group 1: ≤3 AF; Group 2: 4–10 AF; Group 3: ≥11 AF. Retrieval oocyte number, embryo number, pregnancy rate (PR), and implantation rate (IR) of the three groups were compared.*
Results: *A total of 372 cycles were included. Patients in Groups 1, 2, and 3 were statistically different in age (35.3 vs. 31.9 vs. 28.5), Day-3 FSH (14.3 vs. 5.9 vs. 4.1), cancellation (34.4% vs. 2.7% vs. 0.9%), gonadotropin dosage, retrieval oocyte number (2.9 vs. 8.2 vs. 14.5), embryo number (2.2 vs. 6.5 vs. 11.7), PR (11.1% vs. 34.6% vs. 35.0%), and IR (3.0% vs. 8.2% vs. 8.9%).*
Conclusions: *Patients with ≤3 AF have a significantly higher cancellation rate, fewer retrieved oocyte number, and lower PR. Combination of AF counting and basal FSH level increased the sensitivity in predicting the ovarian reserve. Retrieved oocyte number could be predicted by the formula: oocyte = 0.802 × AF + 2.01.*

KEY WORDS: Antral follicle; oocyte; ovarian hyperstimulation; ovarian reserve; transvaginal sonography.

INTRODUCTION

Assessment of the ovarian reserve of infertile women is useful in deciding treatment protocol and predicting treatment outcome. Several methods have been demonstrated in order to detect the ovarian reserve, including Day-3 serum follicle stimulating hormone (FSH) and estradiol (E2) levels (1–3), clomiphene citrate challenge test (4,5), and ovarian antral follicle counting (6,7). Chang *et al.* (6,7) demonstrated that the ovarian response and pregnancy results of patients undergoing assisted reproductive technologies (ARTs) could be predicted by the Day-3 antral follicle (AF) counting. Besides this, no investigator reported the clinical application of AF counting in in-vitro fertilization and embryo transfer (IVF-ET). In this series, using the larger case number, we evaluate the value of AF counting in predicting the clinical outcome after controlled ovarian hyperstimulation (COH) and IVF-ET. To the best of our knowledge, this is the largest series in this aspect.

MATERIALS AND METHODS

All cases at the China Medical College Hospital receiving COH and IVF-ET were included in the prospective investigation. All couples had been evaluated by Day-3 FSH and E2 levels, semen analysis, and hysterosalpingography, if indicated. Patients with polycystic ovarian disease and only one ovary were excluded in this series. Informed consent was signed by all the couples who were included in this series. The study was approved by the Ethical Committee of the China Medical College Hospital. The Institutional Review Board approval has been obtained during the analysis of this series.

Day-3 AFs were measured by the transvaginal ultrasound (4 MHz, Acuson Aspen, Mountain View, California, USA). AFs with diameter 2–10 mm were recorded; AF with diameters >10 mm were excluded. All patients accepted the two times of counting. All cycles were divided into three groups according to

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Table I. Basic Personal Data, Stimulation Response, and Clinical Outcome in Patients with Different Antral Follicle (AF) Numbers

	AF ≤ 3 (n = 32)	AF = 4–10 (n = 223)	AF ≥ 11 (n = 117)
AF No.[a,b]	2.1 ± 0.7	7.2 ± 2.1	16.1 ± 5.3
Age (year)[a,b]	35.3 ± 4.0	31.9 ± 4.2	28.5 ± 3.6
<30	2	61	75
30–37	17	139	41
>38	13	23	1
Basal Day-3 FSH[a,b]	14.3 ± 10.4	5.9 ± 5.4	4.1 ± 3.1
Patients No. (basal FSH)[c]			
<10 mIU/ml	11	196	115
10–25 mIU/ml	14	23	2
>25 mIU/ml	7	4	0
E2 level on hCG administration[a,b]	540.9 ± 195.4	1292.1 ± 607.6	1871.1 ± 756.4
Cancellation or no retrieved oocyte[a]	11 (34.4%)	6 (2.7%)	1 (0.9%)
Gonadotropin dosage (ampule)[a,b]			
<30 years	41.5 ± 4.9	30.9 ± 9.4	26.1 ± 6.2
≥30 years	45.1 ± 10.4	34.4 ± 9.6	28.8 ± 7.3
Retrieval oocyte No.[a,b]	2.9 ± 2.6	8.2 ± 4.1	14.5 ± 6.8
Fertilization rate[d]	69/89 (77.5%)	1442/1827 (78.9%)	1366/1711 (79.8%)
Embryo No.[a,b]	2.2 ± 1.9	6.5 ± 3.7	11.7 ± 6.2
Transferred embryo No.[b,e]	2.2 ± 1.9	4.8 ± 1.4	4.6 ± 1.8
Pregnancy rate[e]			
Per stimulation	2/32 (6.3%)	75/223 (33.6%)	41/117 (35.0%)
Per transfer	2/16 (11.1%)	75/217 (34.6%)	41/117 (35.0%)
Ongoing pregnancy per transfer[e]	1/16 (6.3%)	63/217 (29.0%)	36/117 (30.8%)
Implantation rate[e]	2/67 (3.0%)	81/989 (8.2%)	45/503 (8.9%)

Note. AF: antral follicle.
[a] Group 1 < Group 2 < Group 3 (p value < .005).
[b] Mean ± SD.
[c] p value < .005 (χ^2 test).
[d] Nonsignificant difference between the three groups.
[e] Group 1 < Group 2 and 3 (p value < .005); Nonsignificant difference between Groups 2 and 3.

their AF counting: Group 1: ≤3 AF; Group 2: 4–10 AF; Group 3: ≥11 AF. The numbers of retrieval oocytes and embryos, and clinical characteristics were compared among the three groups (Table I).

The COH with long down-regulation protocol was as previously described (8). In brief, the ovaries were stimulated by gonadotrophin and under the gonadotrophin-releasing hormone agonist (GnRHa) suppression (Leuprolide acetate depot, 1.88 mg, s.c., single dosage, Takeda, Japan) from the previous midluteal phase. During Menstrual Day 3–7, the younger patients (<30 years) accepted three ampules of human menopausal gonadotrophin (HMG, Pergonal, Serono, Rome, Italy) daily. The elder patients (≥30 years) accepted two ampules of HMG and two ampules of follicle stimulating hormone (FSH, Metrodin; Serono, Rome, Italy) daily. On Menstrual Day 7, the serum E2 was measured. If the E2 was ≥100 pg/mL, the daily gonadotrophins were decreased to 2-ampules HMG in the younger patients and to 2-ampules FSH and 1-ampules HMG in the elder patients. The criteria for cancellation included the lower E2 level on Menstrual Day 7 (<50 pg/mL) and the poor follicle growth during the COH.

Gonadotrophin administration continued until two or more follicles ≥18 mm were formed; then the human chorionic gonadotrophin (HCG, 10000 IU, Pregnyl, Organon, Oss, The Netherlands) was administered. Oocytes were transvaginally retrieved 34–36 h later. Oocytes culture, insemination, embryo transfer, and cryopreservation were as previously described (8,9). The luteal phase was supported with HCG (2500 IU/day) on Days 1, 4, and 7 post-ET and progesterone (Duphaston, 15 mg/day, oral, Solvay, Holland) since Day 9 post-ET. A urine pregnancy test was performed 14 days post-ET. Clinical pregnancy was defined as the presence of an intrauterine gestational sac. The SAS system with t test and χ^2 test were used for statistical analyses. A p value of <.05 was considered statistically significant.

RESULTS

A total of 372 cycles for 343 couples were included in this series. The reasons for infertility included

endometriosis ($n = 84$), tubal factors ($n = 73$), male factors ($n = 96$), uterine factors ($n = 15$), ovulatory dysfunction ($n = 28$), and unexplained infertility ($n = 76$). There were nonsignificant difference in the infertility diagnosis and insemination methods (IVF or ICSI) between these three groups. Patients in Groups 1, 2, and 3 were statistically different in the AF number (2.1 ± 0.7 vs. 7.2 ± 2.1 vs. 16.1 ± 5.3), mean age (35.3 ± 4.0 vs. 31.9 ± 4.2 vs. 28.5 ± 3.6), basal Day-3 FSH (14.3 ± 10.4 vs. 5.9 ± 5.4 vs. 4.1 ± 3.1), E2 level on hCG administration (540.9 ± 195.4 vs. 1292.1 ± 607.6 vs. 1871.1 ± 756.4), cancellation or no retrieved oocyte (34.4% vs. 2.7% vs. 0.9%), gonadotropin dosage, retrieval oocyte number (2.9 ± 2.6 vs. 8.2 ± 4.1 vs. 14.5 ± 6.8), and embryo number (2.2 ± 1.9 vs. 6.5 ± 3.7 vs. 11.7 ± 6.2) (all p value $<.05$) (Table I). Patients with AF ≤ 3 had higher cancellation rates, higher gonadotropin dosage, lower E2 response, lower numbers of oocytes and embryos and lower PRs than those of the other two groups.

Patients of three groups were comparable with respect to the fertilization rates (77.5% vs. 78.9% vs. 79.8%). PRs per stimulation or transfer, ongoing PR, and implantation rate (IR) of Group 1 were lower than those of Groups 2 and 3. The similar PR and IR between Groups 2 and 3 were noted (Table I). The regression curve delineates the significant correlation between the numbers of AF and retrieved oocytes ($R2 = 0.563$, $p < .005$) (Fig. 1). Oocyte number could be estimated using AF numbers by the formula, retrieved oocyte $= 0.802 \times \text{AF} + 2.01$.

DISCUSSION

Prediction of ovarian reserve prior to stimulation is important for clinicians. Prediction is important for the clinicians to modify their stimulation protocol. Numerous tests for predicting the ovarian reserve have been reported (4,5). Among these screening tests, basal serum FSH concentration and patient age were most popular, and applicable for predicting the ovarian reserve and clinical outcome (1–3). However, these methods are not completely reliable (10,11). Some women with normal level of basal FSH or of a young age still have poor responses to COH.

Imaging techniques for predicting the ovarian response after hyperstimulation include antral follicle count (6), measurement of ovarian stromal blood flow (12), and ovarian volume measurement (13). In fact, measurement of ovarian flow for predicting the ovarian status is controversial. In our previous survey, we observed that Doppler surveys of ovarian dominant follicle could not provide the useful predictive value in ovarian hyperstimulation and intrauterine insemination (14). The main disadvantage of three-dimensional ultrasonography is its application limitations (not routinely available in many centers, expensive, time-consuming). Furthermore, its value in predicting the ovarian reserve is still controversial. Sharara *et al.* (15) demonstrated the noncorrelation between the age and ovarian volume.

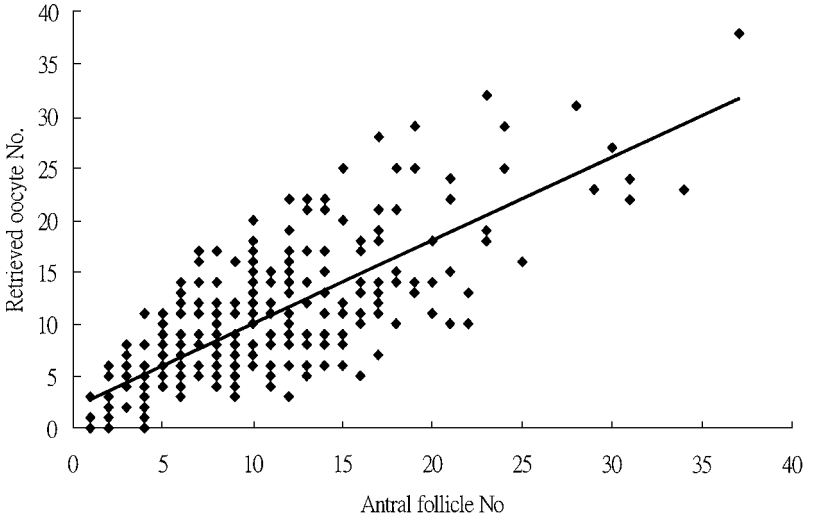

Fig. 1. The relationship and regression curve between antral follicle and retrieved oocyte number (Retrieved oocyte No. $= 0.802 \times$ Antral follicle No $+ 2.01$, $R2 = 0.563$).

Follicle development is a complex and prolonged process. The initiation of follicular growth is independent of gonadotropin stimulation till it progresses to the AF stage (16). After the AF stage, follicle growth or atresia is determined by levels of gonadotropin (17). If gonadotropin stimulation is sufficient, multiple AFs will develop into numerous mature follicles. Because AFs are the main origin of inhibin, decreased AF may be related with decreased inhibin and increased FSH levels (6).

Recently, Scheffer *et al.* (18) demonstrated that the AF numbers are related to the reproductive age. In their longitudinal survey of natural fertile women, the AF count showed a mean decline per year of 4.8% before 37 years of age and 11.7% after 37 years of age. Sharara *et al.* (15) demonstrated that the low AF count is related with diminished ovarian reserve. They measured the ovarian volume and observed that the ovarian volume <3 cm^2 is related to lesser follicle number and higher cancellation rate. In this study, we also observed aged women with fewer AF count. Furthermore, we also noted the correlation between higher basal FSH level and fewer AF count. It suggested that AF count could be used to predict follicle cohort and ovarian reserve.

In this series, we observed that the AF number is useful in predicting ovarian reserve and retrieved oocyte numbers. Patients with AF ≤ 3 required more gonadotropins and were related to the lower E2 levels. By using the formula "oocyte $= 0.802 \times$ AF $+$ 2.01," retrieved oocyte number could be accurately predicted before COH. Therefore, clinicians could modify the gonadotropin type, dosage, and COH protocol (long, short, or ultrashort protocol) before COH and down-regulation.

In this series, we observed that some young women (<30 years) with normal FSH level (<10 mIU/mL) presented lower AF number, poor ovarian response, and high cancellation rate. This suggested that the combination of AF counting with basal FSH level and age increased the sensitivity in assessing ovarian reserve. We also noted that AF number is not related to oocyte quality. However, patients with AF ≤ 3 is related to high cancellation rate, lower PR, and lower IR. This might be because of their lower embryo number for selection and transfer. In women with higher AF number, except for their adequate embryos for transfer, their sufficient embryos could provide the selection of better-quality embryos for transfer.

In conclusion, Day-3 AF counting is useful in predicting the ovarian response, retrieval oocyte number, and embryo number. Patients with ≤ 3 AF have a significantly higher cancellation rate, fewer retrieved oocyte number, and lower PR. By using the formula retrieved oocyte number $= 0.802 \times$ AF number $+$ 2.01, retrieved oocyte number could be accurately predicted. Combination of AF counting with basal FSH level and age increased the sensitivity in predicting the ovarian reserve. This is an easy, simple, reliable and cheap technology to predict the final outcome of ART. Routine Day-3 AF counting is indeed worth more clinical attention and consideration.

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